Supporting Information

I. **Supplementary Methods**

II. **Supplementary Table 1.** Monoclonal antibodies used in the study.

III. **Supplementary Figures.**

Figure S1. Gating strategy for flow cytometry analysis of spleen cell subsets.

Figure S2. Heart allograft placement and mATG lymphoablation induce Mincle expression on myeloid cells. B6.WT mice were transplanted with BALB/c heart allografts with or without mATG treatment (d. 0 and 4), and Mincle expression was measured on d. 8 after transplantation. The gating strategy for myeloid cells is shown in Figure S1. **A.** Representative dot plots. **B.** Quantification of flow cytometry data shown as % of Mincle$^+$ cells within indicated subsets (top) or Mincle$^+$ cells with indicated phenotype per spleen (bottom). n = 4 animals/group; error bars represent SD.

Figure S3. The effect of Mincle deficiency on T cell reconstitution after mATG lymphoablation in skin allograft recipients. B6.WT or B6.Mincle$^{-/-}$ mice were transplanted with BALB/c skin allografts and treated with mATG (d.0, d.4). The numbers of CD4$^+$ and CD8$^+$ T cells per 100 μl of peripheral blood **(A)** or per spleen at d. 28 after transplantation **(B)**. n = 4-5 animals/group; error bars represent SD.

I. Supplementary Methods

Cell viability staining for flow cytometry

Cell viability was determined by staining with either Sytox™-Orange Nucleic Acid Stain or LIVE/DEAD™ Fixable Aqua Dead Cell Stain (both from Invitrogen, Waltham MA) as indicated for individual experiments. Sytox™-Orange was added to antibody-stained cells at 1:15,000 dilution for 20 min at room temperature prior to sample acquisition. LIVE/DEAD™ Aqua was diluted at 1:2,000 in PBS, and added to cell samples for 30 min on ice prior to antibody staining. Cells were then washed with PBS and used for further antibody staining. Live cells were defined as Sytox™-Orange negative or LIVE/DEAD™ Aqua negative (as in **Figure S1**). At least 50,000 live events per sample were collected on an LSR Fortessa X-20 (BD Bioscience). The data was analyzed with FlowJo 10.8.1 (Tree Star Inc., Ashland, OR).

NanoString gene expression analysis

Recipient spleen B cells were isolated on d. 12 posttransplant using EasySep™ Mouse B cell Isolaton Kit (STEMCELL Technologies, Vancouver, BC). All samples contained >95% $B220^+$ cells. RNA isolation and gene expression analysis by NanoString Mouse Immunology Panel (561 genes) were performed as previously published (5). Raw counts normalization, subtraction of background counts, and correction of lane-specific differences were performed with nSolver 3.0 software. Gene expression was normalized to the geometric mean of 12 reference genes followed by normalization to $B220^+$ cells

from naïve B6 mice. The full list of genes up-regulated in B cells by mATG was previously published in (5).

I. **Supplementary Table 1.** Monoclonal antibodies used in the study

Reactivity	**conjugate**	**clone**	**source**	**catalog number**
Mouse CD4	PE	GK1.5	BD Biosciences	553730
Mouse CD4	FITC	RM4-5	BD Biosciences	553047
Mouse CD8α	APC	53-6.7	BD Biosciences	553035
Mouse B220	PE-Cy7	RA3-6B2	BD Biosciences	552772
Mouse Mincle	-	4A9	MBL	D292-3
Rat IgG1	APC	R1-12D10	Invitrogen	17-4812-82
Mouse IL-1β	PE	NJTEN3	Invitrogen	12-7114-82
Mouse IL-6	PE	MP5-20F3	BD Biosciences	562050
Mouse TNFα	PE	MP6-XT22	Invitrogen	14-7321-81
Mouse CD11b	APC	M1-70	Invitrogen	17-0112-83
Mouse CD11c	FITC	N418	Invitrogen	11-0114-85
Mouse CD4	-	GK1.5	BioXCell	BE0003-1
Mouse CD4	-	YTS191	BioXCell	BE0119
Mouse CD154	-	MR-1	BioXCell	BP0017-1
Mouse CD40	-	FGK4.5	BioXCell	BP0016-2

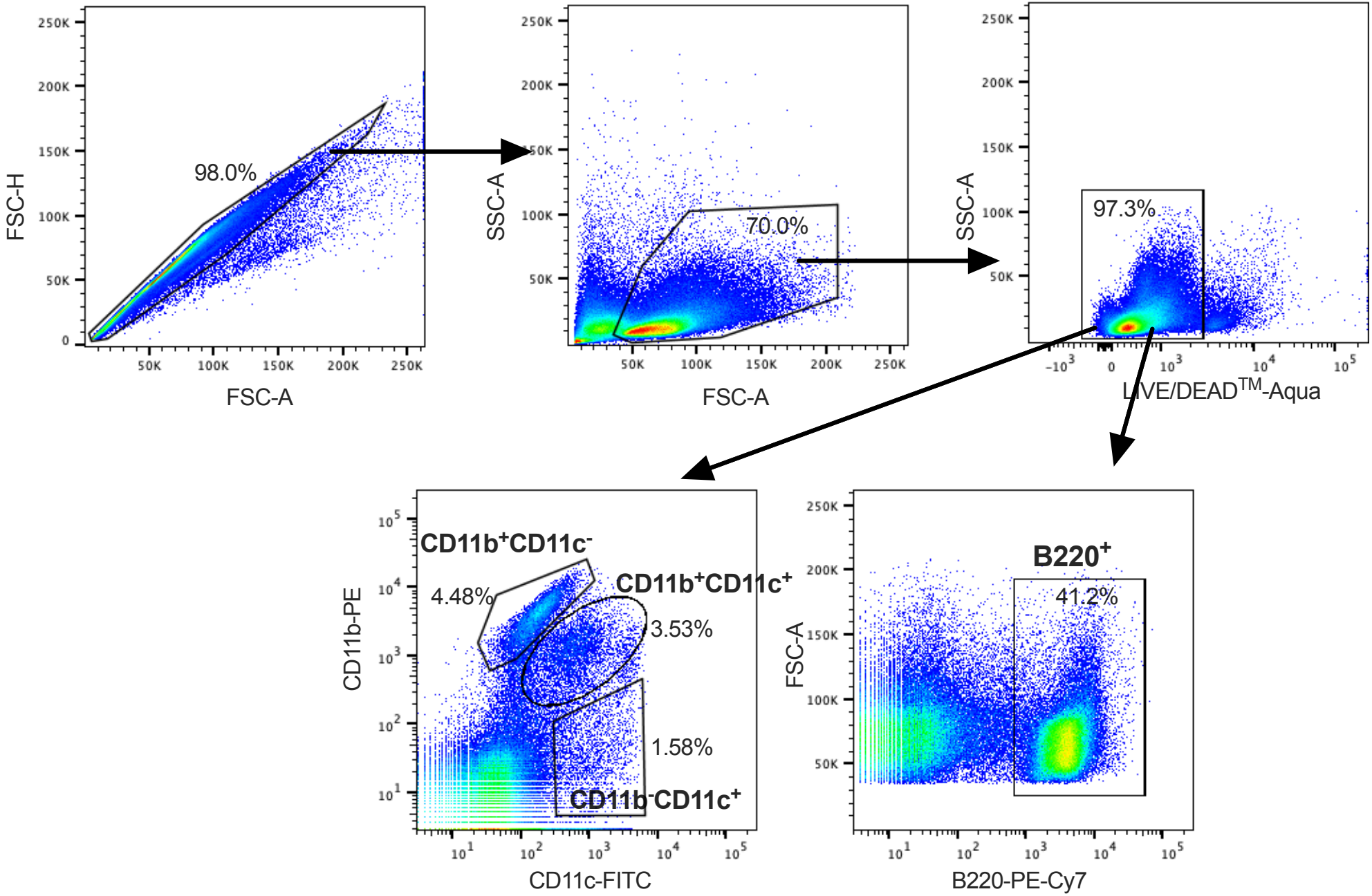

Figure S1. Gating strategy for flow cytometry analysis of spleen cell subsets.

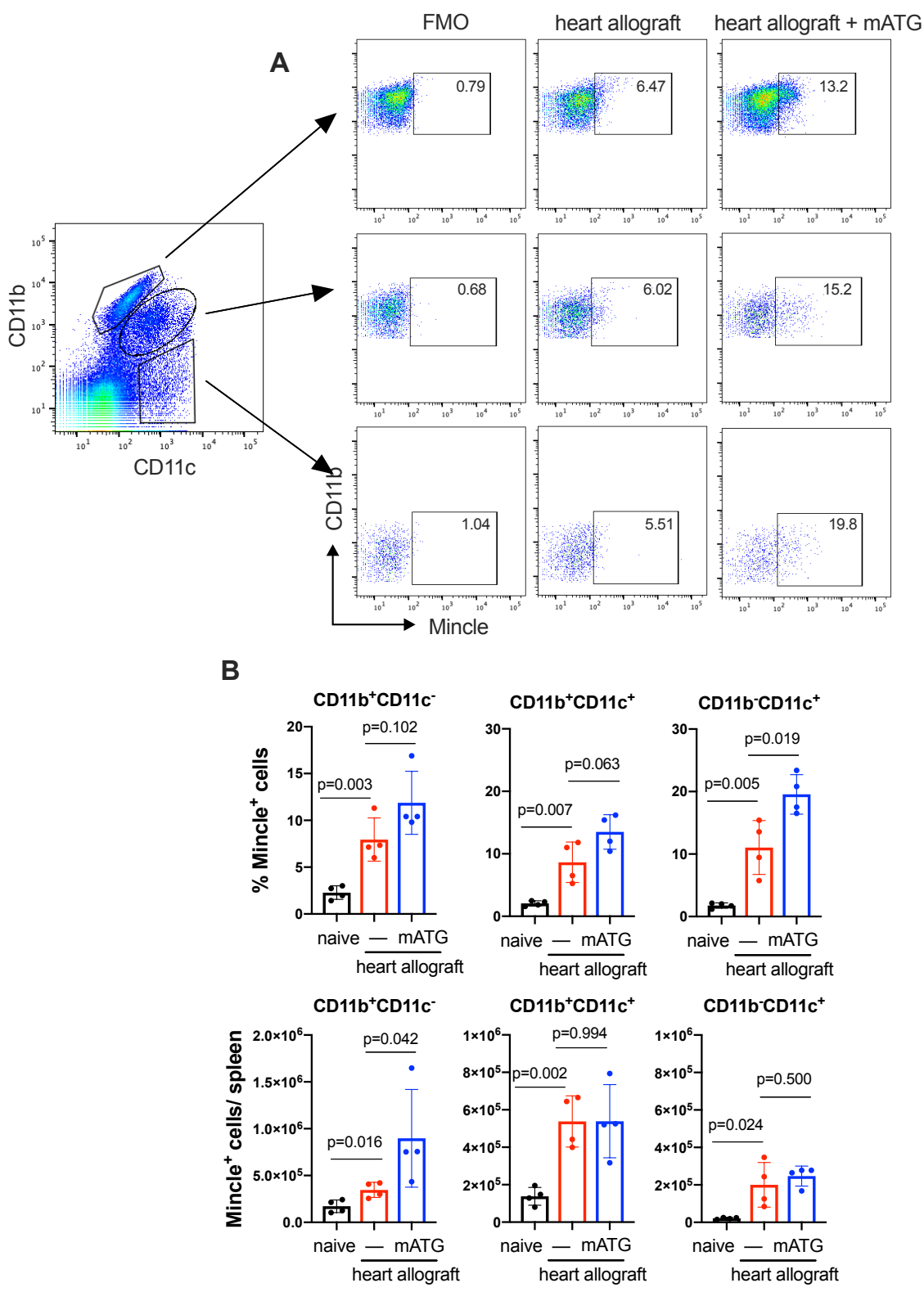

Figure S2. Heart allograft placement and mATG lymphoablation induces Mincle expression on myeloid cells. B6 mice were transplanted with BALB/c heart allografts with or without mATG treatment (d. 0 and 4), and Mincle expression was measured on d. 8 after transplantation. Gating strategy for myeloid cells is shown in Figure S1. **A.** Representative dot plots. **B.** Quantification of flow cytometry data shown as % of Mincle$^+$ cells within indicated subsets (top) or Mincle$^+$ cells with indicated phenotype per spleen (bottom). N = 4 animals/group, error bars represent SD.

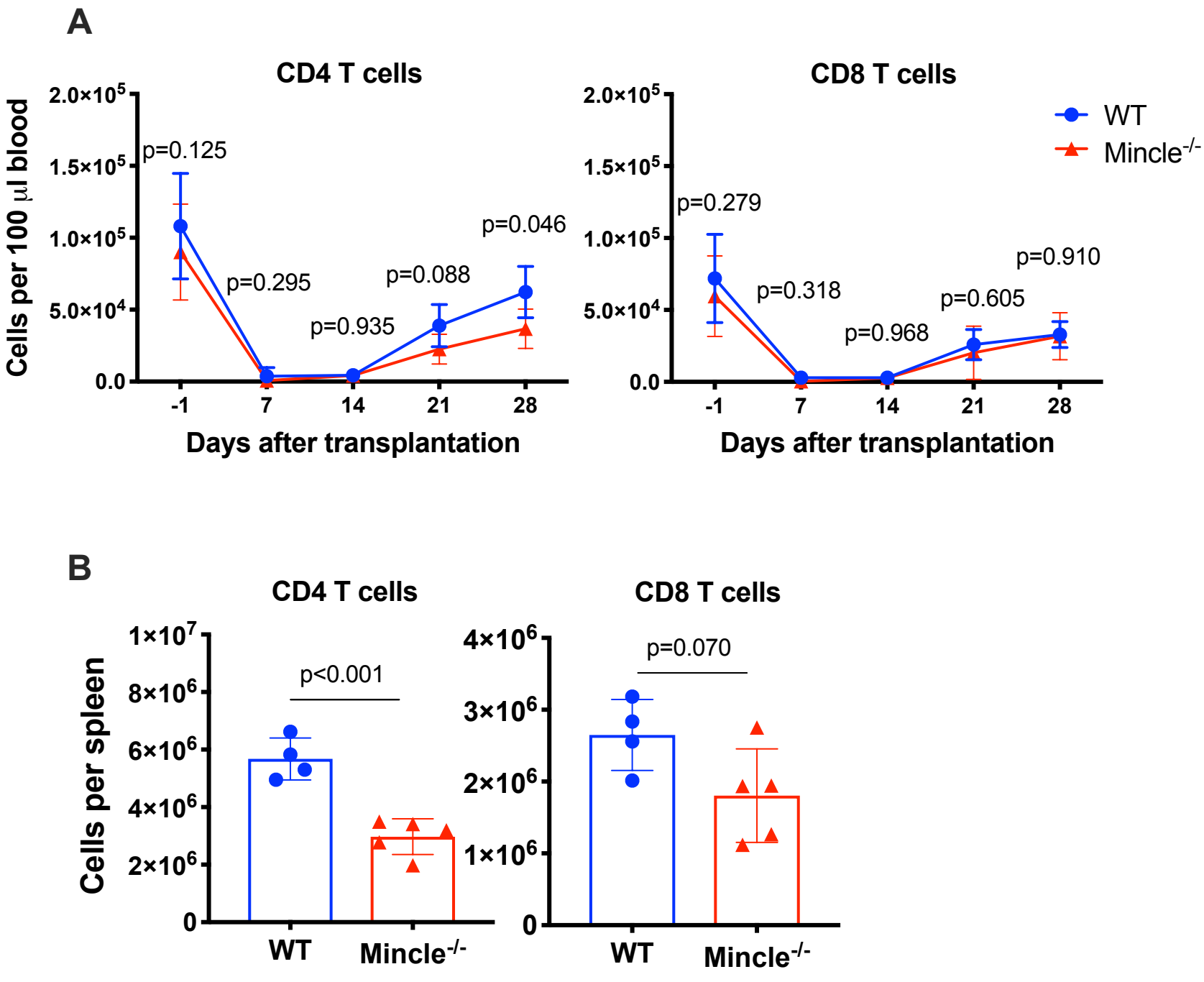

Figure S3. The effect of Mincle deficiency on T cell reconstitution after mATG lymphoablation in skin allograft recipients. B6.WT or B6.Mincle$^{-/-}$ mice were transplanted with BALB/c skin allografts and treated with mATG (d.0, d.4). The numbers of CD4^{+} and CD8^{+} T cells per 100 μl of peripheral blood (A) or per spleen at d. 28 after transplantation (B). n = 4-5 animals/group; error bars represent SD.